Claims

Please substitute the following claims for the set currently of record:

1. (Currently amended) A method for detecting eubacteria and determining species of said eubacteria in a sample, comprising:

performing a real-time polymerase chain reaction (PCR) using a sample which may comprise template DNA of a first species of eubacteria, wherein the PCR employs primers and at least two fluorogenic probes,

wherein the primers are complementary to two flanking regions of a *S. aureus* 16S rRNA gene, wherein the two flanking regions flank a segment of the *S. aureus* 16S rRNA gene comprising a conserved region and a first divergent region,

wherein the conserved region comprises at least 18 contiguous nucleotides which are at least 80% identical among at least 10 eubacterial species,

wherein the first divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from a second divergent region found in a *Bradyrhizobium japonicum* 16S rRNA gene;

wherein each of the fluorogenic probes comprises a reporter dye and a quencher dye,

wherein a first of the two fluorogenic probes is complementary to the conserved region of the *S. aureus* 16S rRNA gene and the second of the two fluorogenic probes is complementary to a third divergent region of the first species of eubacteria, wherein the third divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from the second divergent region found in a *Bradyrhizobium japonicum* 16S

rRNA gene,

wherein the reporter dyes of the first and the second probes have non-overlapping emission spectra;

monitoring fluorescence emissions of the reporter dyes;

detecting presence of eubacteria in the sample when emissions characteristic of the reporter dye of the first probe are detected; and

determining presence of the first species of eubacteria in the sample when emissions characteristic of the reporter dye on the second probe are detected.

2. (Currently amended) The method of claim 1 wherein a third fluorogenic probe is employed in the real-time PCR, wherein the third fluorogenic probe is complementary to a fourth divergent region of 16S rRNA gene in a second species of eubacteria, wherein the fourth divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from the second divergent region found in a *Bradyrhizobium japonicum* 16S rRNA gene, and wherein the reporter dye of the third fluorogenic probe has a non-overlapping emission spectrum from the reporter dyes of the first and second probes; wherein the presence of the second species of eubacteria is determined when emissions characteristic of the reporter dye on the third fluorogenic probe are detected.

3. (Currently amended) The method of claim 2 wherein a fourth fluorogenic probe is employed in the real-time PCR, wherein the fourth fluorogenic probe is complementary to a fifth divergent region of 16S rRNA gene in a third species of eubacteria, wherein the fifth divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from the second divergent region found in a *Bradyrhizobium japonicum* 16S rRNA gene, and wherein the reporter dye of the fourth fluorogenic probe has a non-overlapping emission spectrum from the reporter dyes of the first, second, and third probes; wherein the presence of the third species of eubacteria is determined when emissions characteristic of the reporter dye on the fourth

fluorogenic probe are detected.

4. (Previously presented) The method of claim 1 wherein the segment of *S. aureus* 16S rRNA gene comprises nucleotides 890 to 912 and 1033 to 1051 as shown in SEQ ID NO: 1 and 2, respectively.

5. (Previously presented) The method of claim 1 wherein the conserved region of *S. aureus* 16S rRNA gene comprises nucleotides 1002 to 1024 as shown in SEQ ID NO: 3.

6. (Previously presented) The method of claim 1 wherein the first divergent region comprises nucleotides 945 to 978 of *S. aureus* 16S rRNA gene as shown in SEQ ID NO: 4.

7. (Original) The method of claim 1 wherein the sample is a treated blood sample.

8. (Original) The method of claim 7 wherein the blood sample is from a patient suspected of systemic bacteremia.

9. (Original) The method of claim 7 wherein the sample was treated to extract DNA from cells.

10. (Original) The method of claim 1 wherein the sample is urine.

11. (Original) The method of claim 1 wherein the sample is cerebrospinal fluid.

12. (Original) The method of claim 1 wherein the primers comprise primers p890F and p1033R (SEQ ID NO: 1 and 2, respectively.)

13. (Original) The method of claim 1 wherein the segment amplified is at least 125 bp.

14. (Original) The method of claim 1 wherein the segment amplified is at least 150 bp.

15. (Original) The method of claim 1 wherein the segment amplified is at least 160 bp.

16. (Currently amended) A method for detecting eubacteria and determining species of said eubacteria in a sample, comprising:

filtering a real-time PCR reaction mixture to remove double stranded DNA contaminants having a length of > 125 bp to form a filtrate, wherein the PCR reaction mixture comprises primers and at least two fluorogenic probes,

wherein the primers are complementary to two flanking regions of a *S. aureus* 16S rRNA gene,

wherein the two flanking regions flank a segment of the *S. aureus* 16S rRNA gene comprising a conserved region and a first divergent region,

wherein the conserved region comprises at least 18 contiguous nucleotides which are at least 80% identical among at least 10 eubacterial species,

wherein the first divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from a second divergent region of *Bradyrhizobium japonicum* 16S rRNA gene,

wherein each of the probes comprises a reporter dye and a quencher dye,

wherein a first of the two fluorogenic probes is complementary to the conserved region of *S. aureus* 16S rRNA and the second of the two fluorogenic probes is complementary to a third divergent region of a first eubacterial species, wherein the third divergent region comprises at least 10

contiguous nucleotides and differs by at least 3 nucleotides from a second divergent region found in a *Bradyrhizobium japonicum* 16S rRNA gene, wherein the reporter dyes of the first and the second probes have non-overlapping emission spectra;

adding a sample which may comprise template DNA of the first species of eubacteria to the filtrate to form a complete reaction mixture;

amplifying template DNA of the first species of eubacteria which may be present in the complete reaction mixture;

monitoring fluorescence emissions of the reporter dyes;

detecting presence of eubacteria in the sample when emissions characteristic of the reporter dye of the first probe are detected; and

determining presence of the first species of eubacteria in the sample when emissions characteristic of the reporter dye on the second probe are detected.

17. (Currently amended) The method of claim 16 wherein a third fluorogenic probe is present in the real-time PCR reaction mixture, wherein the third fluorogenic probe is complementary to a fourth divergent region of a 16S rRNA gene in a second species of eubacteria, wherein the fourth divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from the second divergent region found in a *Bradyrhizobium japonicum* 16S rRNA gene, and wherein the reporter dye of the third fluorogenic probe has a non-overlapping emission spectrum from the first and second probes, wherein the presence of the second species of eubacteria is determined when emissions characteristic of the reporter dye on the third fluorogenic probe are detected.

18. (Currently amended) The method of claim 17 wherein a fourth fluorogenic probe is present in the real-time PCR reaction mixture, wherein the fourth fluorogenic probe is complementary to a fifth divergent region of a 16S rRNA gene in a third species of eubacteria,

wherein the fifth divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from the second divergent region found in a *Bradyrhizobium japonicum* 16S rRNA gene, and wherein the reporter dye of the fourth fluorogenic probe has a non-overlapping emission spectrum from the first, second, and third probes; wherein the presence of the third species of eubacteria is determined when emissions characteristic of the reporter dye on the fourth fluorogenic probe are detected.

19. (Previously presented) The method of claim 16 wherein the segment comprises nucleotides 890 to 912 and 1033 to 1051 as shown in SEQ ID NO: 1 and 2, respectively.

20. (Previously presented) The method of claim 16 wherein the conserved region comprises nucleotides 1002 to 1024 of *S. aureus* 16S rRNA gene as shown in SEQ ID NO: 3

21. (Previously presented) The method of claim 16 wherein the first divergent region comprises nucleotides 945 to 978 of *S. aureus* 16S rRNA gene as shown in SEQ ID NO: 4

22. (Original) The method of claim 16 wherein the sample is a treated blood sample.

23. (Original) The method of claim 22 wherein the blood sample is from a patient suspected of systemic bacteremia.

24. (Original) The method of claim 22 wherein the sample was treated to extract DNA therefrom.

25. (Original) The method of claim 16 wherein the sample is urine.

26. (Original) The method of claim 16 wherein the sample is cerebrospinal fluid.

27. (Original) The method of claim 16 wherein the primers comprise primers p890F and p1033R (SEQ ID NO: 1 and 2, respectively.)

28. (Original) The method of claim 16 wherein the segment amplified is at least 125 bp.

29. (Original) The method of claim 16 wherein the segment amplified is at least 150 bp.

30. (Original) The method of claim 16 wherein the segment amplified is at least 160 bp.

31. (Original) The method of claim 16 wherein the conserved region is at least 80% identical among at least 14 eubacterial species.

32. (Original) The method of claim 1 wherein the conserved region is at least 80% identical among at least 14 eubacterial species.

33. (Original) The method of claim 1 wherein the first divergent region comprises at least 15 contiguous nucleotides.

34. (Original) The method of claim 1 wherein the first divergent region comprises at least 20 contiguous nucleotides.

35. (Original) The method of claim 1 wherein the first divergent region comprises at least 25 contiguous nucleotides.

36. (Original) The method of claim 1 wherein the first divergent region comprises at least 30 contiguous nucleotides.

37. (Original) The method of claim 16 wherein the first divergent region comprises at least 15 contiguous nucleotides.

38. (Original) The method of claim 16 wherein the first divergent region comprises at least 20 contiguous nucleotides.

39. (Original) The method of claim 16 wherein the first divergent region comprises at least 25 contiguous nucleotides.

40. (Original) The method of claim 16 wherein the first divergent region comprises at least 30 contiguous nucleotides.

41. (Original) The method of claim 1 wherein the first divergent region differs by at least 4 nucleotides from the second divergent region.

42. (Original) The method of claim 16 wherein the first divergent region differs by at least 4 nucleotides from the second divergent region.

43.-52. (Cancelled)